■ Interagency Ecological Program for the San Francisco Estuary ■



# IEP NEWSLETTER

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# OF INTEREST TO MANAGERS

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One of the biggest highlights of this issue of the IEP Newsletter is Brett Harvey's (DWR) historical account of the length criteria used to separate different races of juvenile Chinook salmon in the Central Valley. These criteria are widely used to determine endangered species "take" from sampling and water diversions, making them a key management issue. Until now, there had never been a thorough review of how the length criteria had been developed. Hence, this represents an important foundation to evaluate future improvements in race identification methods.

An article by Sara Blaser and colleagues (SFSU) provides new information about the sensitivity of phytoplankton to herbicides used in the San Francisco estuary. Herbicides represent a possible contributing factor to the chronically low phytoplankton levels in much of the estuary. Their results indicate that two herbicides may intermittently be at high enough levels to affect primary production.

Alpa Wintzer and Mariah Meek (UCD) provide species descriptions and ecological information about jelly-fish polyps that occur in the upper estuary. These invasive jellyfish are an emerging concern due to their periodic high densities and food web effects.

Caily Nelson and colleagues (DWR) contributed an article about mysid shrimp in the Cache Slough Complex, a focus of proposed habitat restoration efforts in the Delta. Mysid shrimp are a major food source for several of the key fishes in the estuary. Their results show that mysids occur at relatively high levels in Cache Slough Complex during times of the year when other parts of the Delta have low food resources. This supports the idea that habitat restoration could have food web benefits to the estuary.

Jason Dubois and Marty Gingras (DFG) summarize a new method to estimate the abundance of white sturgeon, an important sport fish and a target for restoration activities. Their initial results suggest that the alternative method of estimating white sturgeon abundance is precise, relatively fast, and reasonably accurate.

Finally, Rene Reyes and Bandon Wu (USBR) provide an account of spawning by the alien smelt wakasagi in Los

Vaqueros reservoir. Wakasagi represent a continuing concern for the native delta smelt because the two can hybridize and wakasagi may compete with delta smelt for resources. Although wakasagi have been present in the Delta for years, this is the first documented egg collection for this species. An understanding of their spawning behavior and preferences may help in the development of management practices to reduce the negative effects of wakasagi.

# IEP QUARTERLY HIGHLIGHTS

## Diuron and Imazapyr Herbicides Impact Estuarine Phytoplankton Carbon Assimilation: Evidence from an Experimental Study

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#### **Abstract**

Herbicides applied in the San Francisco Estuary (SFE) watershed have the potential to be flushed into the estuary where they may negatively affect aquatic organisms. Two herbicides of concern in the SFE are diuron, which is heavily used and persistent in the environment, and imazapyr, which is applied to lands immediately adjacent to and directly within tidal marsh habitats in the SFE to control invasive plants. At present, little is known about the concentrations of these herbicides within the SFE or their potential impact on pelagic primary production. This study investigated the effects of additions of either diuron or imazapyr on carbon assimilation by both a natural assemblage of phytoplankton in the SFE and by a cultured diatom (Thalassiosira pseudonana) during both acute (T = 0 hr) and chronic (T = 48 hr) exposure. Results from 5 diuron experiments using SFE phytoplankton showed a decrease in carbon assimilation number during both acute and chronic exposure. Compared to the control, acute exposure experiments showed a 19, 33 and 65% decrease in carbon assimilation for 1, 2 and 5 ug L<sup>-1</sup> treatments, respectively. Chronic exposure experiments showed a stronger effect; a 31, 62 and 84% decline for 1, 2 and 5 µg L<sup>-1</sup> treatments, respectively, compared to the control. An experiment with T. pseudonana corroborated these results for acute exposure. Diuron has been measured at concentrations up to 2.14 µg L<sup>-1</sup> in the northern SFE, suggesting that at times in the northern estuary diuron concentrations may be sufficient to negatively impact

phytoplankton productivity. Results from 3 imazapyr experiments showed no difference in carbon assimilation after acute exposure, but in chronic exposure experiments, carbon assimilation declined by 16, 53 and 80% (compared to the control) with additions of 5, 15 and 50 mg L<sup>-1</sup> imazapyr. Imazapyr concentrations up to 4.2 mg L<sup>-1</sup> have been measured in the SFE in waters directly adjacent to sites of application during the first tidal flush after application, but this likely dilutes rapidly with time from application. The cultured diatom also responded negatively with chronic exposure to imazapyr with a severe decrease in carbon assimilation number.

#### Introduction

San Francisco Estuary, the largest estuary on the west coast of the United States, is heavily influenced by the surrounding metropolitan and agricultural areas. Anthropogenic contaminants are flushed from the land into the estuary where they have the potential to negatively affect aquatic organisms. The Sacramento and San Joaquin rivers carry nearly 50% of California's runoff to the SFE (Jassby, 2008). The SFE is classified as a low chlorophyll estuary despite relatively high inorganic nutrient concentrations (Cloern, 2001) and one contributing factor for the low chlorophyll may be herbicides that enter the estuary. In the SFE watershed, 5.5 million pounds of herbicide are applied annually (Kuivila et al., 1999); some of this likely reaches the estuary where it may affect estuarine primary production.

Contamination by one such herbicide, diuron (N-(3,4dichlorophenyl)-N) is of toxicological concern in the northern SFE where it is widely used and persistent in the environment (Williams et al., 2009). Diuron is a nonselective phenylurea herbicide which blocks the electron transfer at Photosystem II (Giacomazzi & Cochet, 2004) and is used in both agricultural and urban weed control as well as in antifouling paints for boats. In 2006, over 175,000 kg (active ingredient) of diuron was applied to land in the SFE Watershed (California Department of Pesticide Regulation 2008 as cited by Kuivila & Hladik, 2008). Weed control of rights-of-ways accounted for 42% of diuron application in the California Central Valley in 2000 (Miller et al., 2002). Diuron is resistant to both photodegradation (Giacomazzi & Cochet, 2004) and biodegradation (Thomas et al., 2002). Despite the widespread use of diuron around the SFE, little is known about the concentrations that occur in the SFE and the contamination impact on pelagic primary production. A preliminary

study by Edmunds et al. (1999) noted that localized occurrences of elevated diuron might reduce estuarine phytoplankton productivity in the SFE. Kuivila et al. (1999), found widespread occurrences of diuron (along with other herbicides) in the San Francisco - San Joaquin Delta with diuron concentrations reaching 2.14  $\mu$ g L<sup>-1</sup> (Table 1). A recent review of diuron criteria for the Sacramento River (Fojut et al., 2010) highlights the lack of more detailed studies of phytoplankton and herbicide interactions such as dose-response curves or estimates of threshold concentrations (i.e. concentration below which there is no effect).

Imazapyr is another herbicide of concern in the SFE. Although it is not as widely used as diuron in the watershed, it was recently approved for aquatic use in California (Hogle et al., 2007). Imazapyr is currently applied directly within tidal marsh throughout the SFE to control the invasive smooth cordgrass, *Spartina alterniflora* (Pless, 2005). It is also used to control invasive *Lepidium latifolium* (pepperweed) in the tidal marsh and marsh-upland ecotone at Rush Ranch (Ferner, 2009, personal communication, see "Notes"). In 2006, more than 9,600 kg of imazapyr (active ingredient) were applied to lands in the SFE watershed (California Department of Pesticide Regulation 2008 as cited by Kuivila and Hladik, 2008). Imazapyr is a non-selective herbicide used for annual and perennial broadleaf weeds and grasses, woody species

and aquatic weed species (Hurley et al., 2007). In addition to invasive plant control, imazapyr is used for weed control of rights-of-way, in forest management, and agriculture (Fisher et al., 2003). Imazapyr is highly soluble in water and photodegradation is the primary method of breakdown in aquatic systems (Fisher et al., 2003). Laboratory studies suggest a half life of 5.3 days for imazapyr due to photodegradation (Fisher et al., 2003, Hurley et al., 2007) although photodegradation may be slowed in estuaries such as the SFE, due to high turbidity (Fisher et al., 2003). As with diuron, little research has been reported that evaluates the effect of imazapyr on pelagic primary production.

The overall aim of this study was to investigate the effects of diuron and imazapyr herbicides on phytoplankton carbon assimilation in the SFE. Specifically, experiments were conducted in pursuit of two objectives: (1) to develop a phytoplankton assimilation number doseresponse curve for each herbicide using natural phytoplankton communities as well as cultured diatoms; and (2) to determine primary production responses after acute (immediate) and chronic (48 hour) exposure to the herbicides.

Table 1 Diuron and imazapyr concentrations recorded in San Francisco Bay.

Date	Location	Concentration	Source		
Diuron					
5/27/1997	Paradise Cut	1.01 µg L-1	Kuivila et al., 1999		
11/11/1997	French Camp Slough	2.14 µg L-1	Kuivila et al., 1999		
2/28/2008	Cache Slough	1.21 µg L-1	Fong, unpublished <sup>a</sup>		
3/13/2008	Cache Slough	0.74 μg L-1	Fong, unpublished <sup>a</sup>		
3/28/2008	Cache Slough	0.97 μg L-1	Fong, unpublished <sup>a</sup>		
10/23/2009	Central Bay (RTC Pier)	0.03 µg L-1	McMillin, unpublished <sup>b</sup>		
10/23/2009	Rio Vista	0.02 µg L-1	McMillin, unpublished <sup>b</sup>		
lmazapyr					
2006	Alameda Flood Cont. Chan.	0.5 mg L-1	Kerr, 2007		
9/9/2010	Rush Ranch	4.2 mg L-1	Ferner 2011, unpublished <sup>c</sup>		

<sup>&</sup>lt;sup>a</sup>Fong, personal communication, see "Notes" <sup>b</sup>McMillin, personal communication, see "Notes"

<sup>&</sup>lt;sup>c</sup> Ferner, 2011, personal communication, see "Notes"

#### **Methods and Materials**

Five experiments were conducted between May 2009 and May 2010, to test the effect of diuron additions on primary production of a mixed natural phytoplankton communities collected in Central San Francisco Bay (Table 2). For each experiment, 120 L of surface water was collected with a clean bucket from the seawall at the Romberg Tiburon Center, San Francisco State University (Figure 1). Water was dispensed into an acid-washed, 200-L HDPE container and homogenized. Aliquots were then poured into a series of 10-L LDPE 'cubitainers' (experimental enclosures). A stock solution of diuron (99.9% purity. Chem Service, Inc) was made fresh for each experiment according to Williams et al. (2009) where 10 mg diuron was dissolved in 1 mL methanol. Distilled water was then added to 100 mL resulting in a stock solution of 1 mg L<sup>-1</sup> diuron. This herbicide stock was then added to the cubitainers to make the desired concentrations up to 5 µg L<sup>-1</sup>. The diuron concentration range was picked to reflect ambient concentrations of diuron in the SFE, which have been measured as high as 2.14 µg L<sup>-1</sup> (Table 1). One control cubitainer received no diuron or methanol addition. An additional control cubitainer received methanol alone. Water samples from the experiment in October 2009 were sent to the Department of Fish and Game, Pesticide Investigation Unit for analysis of diuron concentrations in the treatments; the background level of diuron in the Central

Bay was 0.03 μg L<sup>-1</sup> (Table 1; McMillin, personal communication, see "notes").

Three experiments testing the effect of imazapyr were conducted between March and May 2010 (Table 2). For each experiment, 80 L of surface Central Bay water was collected in the same manner as for the diuron experiments and divided into different 4-L LDPE 'cubitainers' for each imazapyr treatment along with one control container (no herbicide addition). Imazapyr (in the form of Habitat®) was added to make the desired concentrations (up to 50 mg L<sup>-1</sup>). Imazapyr was supplied in solution so that no methanol control was needed.

Two exposure experiments (one with diuron, the other with imazapyr) were conducted in June 2010 (Table 2) to confirm that the effects seen in experiments using the natural community were a direct response to the herbicides and not due to a synergistic response from an unidentified stressor in SF Bay water. A monoculture of the diatom Thalassiosira pseudonana was grown in water collected from offshore Monterey Bay, which was assumed to not contain appreciable ambient herbicides. Inorganic nutrients (F/2; Guillard & Ryther, 1962) were added and the culture was grown under artificial fluorescent light (320 umol photon m<sup>-2</sup> s<sup>-1</sup>) at 17 °C. The diuron experiment was conducted for one day to examine acute effects on T. pseudonana while the imazapyr experiment was carried out for 48 hours and measured both acute and chronic effects.

Table 2 Dates of experiments conducted for this study with information about herbicide used, type of phytoplankton community (natural SFE phytoplankton or cultured *Thalassiosira*), duration of experiment and concentration of herbicide used. Acute = sampled immediately after herbicide exposure. Chronic = sampled after 48 hour exposure.

Date	Algae Composition	Duration	Herbicide Addition Concentrations		
Diuron					
May 2009	Mixed Natural	Chronic	0 - 5 μg L-1		
July 2009	Mixed Natural	Acute	0 - 5 μg L-1		
August 2009	Mixed Natural	Acute	0 - 5 μg L-1		
October 2009	Mixed Natural	Chronic	0 - 5 μg L-1		
May 2010	Mixed Natural	Chronic	0 - 5 μg L-1		
June 2010	Thalassiosira pseudonana	Acute	0 - 5 μg L-1		
lmazapyr					
March 2010	Mixed Natural	Chronic	0 - 50 mg L-1		
April 2010	Mixed Natural	Chronic	0 - 50 mg L-1		
May 2010	Mixed Natural	Chronic	0 - 50 mg L-1		
June 2010	Thalassiosira pseudonana	Chronic	0 - 50 mg L-1		

Immediately after the herbicide was added, samples were removed from all cubitainers to assess the potential for acute toxicity. Primary production was measured in all cubitainers and the control container was also sampled for analysis of dissolved inorganic carbon (DIC) and chlorophyll-a concentrations. After sampling, the sealed cubitainers were placed in outdoor tanks under ambient temperature and light conditions. After 48 hours the cubitainers were subsampled again to determine the effects of chronic exposure; measurements of primary productivity rates and concentrations of DIC and chlorophyll-a.

Primary production was measured with carbon-14 using a modified JGOFS (1996) protocol in light/dark bottles. Triplicate 160-mL clear polycarbonate bottles were filled with water from each treatment container and inoculated with 6.4 µCi NaH<sup>14</sup>CO<sub>3</sub>. A dark bottle was also collected from the control and inoculated with <sup>14</sup>C. All bottles were incubated for 6 hours in flowing baywater in order to maintain ambient water temperature, and under a single layer of window screening to reduce ambient irradiance by 50%. At the end of the incubation period, sample water from the bottles was filtered onto 25-mm diameter Whatman GF/F filters with nominal pore size of 0.7 μm, acidified with 250 μL 10% hydrochloric acid to remove unincorporated H<sup>14</sup>CO<sub>3</sub>, and allowed to dry for 24 hours in 8-mL scintillation vials. Once the filters were dry, 6 mL of Optiphase 'Hisafe' 3 scintillation cocktail was added to each vial. Samples were kept in the dark for 24 hours and the radioactivity counted on a Winspectral Guardian 1414 scintillation counter (Perkin Elmer). Primary production (PP) rates were calculated with the following equation:

$$(\mu g C L^{-1} h^{-1}) = \frac{DPM_{Light} - DPM_{Dark}}{DPM_{TA} \bullet T} \bullet 1.05 DIC$$

Where:

 $DPM_{Light}$  = Light bottle count in disintegrations per minute

 $DPM_{Dark}$  = Dark bottle count in disintegrations per minute

 $DPM_{TA}$  = Total activity added to bottle

T = Incubation time (hours)

DIC = Dissolved inorganic carbon concentration in sample ( $\mu$ gC L<sup>-1</sup>)

An isotope discrimination factor of 1.05 was added (Falkowski & Raven, 2007)

Dissolved inorganic carbon concentration was measured according to the methods described by Friederich et al. (2002) and Parker et al. (2006) on a MBARI Mark II

clone DIC analyzer with a Li-Cor CO<sub>2</sub> Analyzer (model LI-6252).

Carbon assimilation was reported as phytoplankton assimilation number, PBM (primary production normalized for chlorophyll-a), to correct for differences in phytoplankton standing stock between experiments. Extracted chlorophyll-a was measured according to the methods of Arar & Collins (1992) modified for estuarine samples by Wilkerson et al. (2006); 100 mL samples were filtered through a 25-mm diameter Whatman GF/F filter under low vacuum pressure ( $\leq 0.02$  MPa). Filters were then placed in glass culture tubes and stored in the freezer for up to 2 weeks until analysis. Prior to analysis, 8 mL of 90% acetone was added to the culture tubes to extract chlorophyll-a from cells and the tubes were returned to the freezer for 24 hours. After 24 hours the extracted chlorophyll-a was measured on a Turner 10-AU fluorometer. Chlorophyll-a concentrations were calculated from the raw fluorescence values based on the calibration of chlorophyll-a standards (Turner Designs Standard Chlorophyll-a).

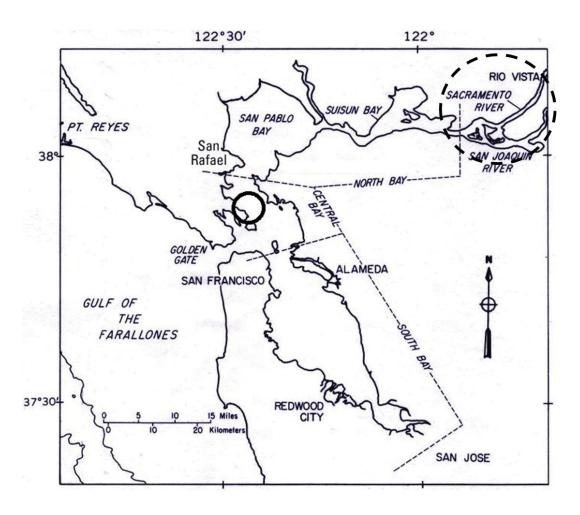


Figure 1 Map of San Francisco Bay. Water was collected in Central Bay (area in circle) for experiments in 2009 and 2010. Measurements of ambient diuron concentrations (Table 1) are for the San Francisco Delta (dashed line circle).

#### Results

With the addition of the herbicide diuron phytoplankton assimilation numbers declined for both acute and chronic effects during all experiments (Figure 2). During acute tests, the average  $P_{\ M}^{B}$  in the control treatment from five experiments was  $6.75\pm0.88~\mu gC(\mu gChl-a)^{-1}hr^{-1}$  (average  $\pm$  SD, n=13). Average  $P_{\ M}^{B}$  declined to  $5.50\pm0.61~\mu gC(\mu gChl-a)^{-1}hr^{-1}$  (n= 13) with the addition of +1  $\mu g~L^{-1}$  diuron, to  $4.51\pm0.78~\mu gC(\mu gChl-a)^{-1}hr^{-1}$  (n= 12) with the addition of +2  $\mu g~L^{-1}$  diuron and to  $2.35\pm1.04~\mu gC(\mu gChl-a)^{-1}hr^{-1}$  (n= 13) with an addition of +5  $\mu g~L^{-1}$  diuron, representing a 19, 33 and 65% decrease in  $P_{\ M}^{B}$  respectively compared to the control. During chronic exposure tests, the average  $P_{\ M}^{B}$  in the control treatment was  $8.82\pm0.77~\mu gC(\mu gChl-a)^{-1}hr^{-1}$  (n=7). Average  $P_{\ M}^{B}$ 

decreased to  $6.08\pm0.86~\mu g C (\mu g Chl-a)^{-1} hr^{-1} (n=7)$  with the addition of +1  $\mu g L^{-1}$  diuron, to  $3.393\pm0.24~\mu g C (\mu g Chl-a)^{-1} hr^{-1} (n=6)$  with the addition of +2  $\mu g L^{-1}$  diuron and to  $1.39\pm0.76~\mu g C (\mu g Chl-a)^{-1} hr^{-1} (n=7)$  with an addition of +5  $\mu g L^{-1}$ , representing a 31, 62 and 84% decline in  $P^B_M$  respectively compared to the control. The phytoplankton response in the methanol control (n=10) was the same as the control (n=13) at t=0 hours (p-value=0.71) but was significantly higher (11%) than the control after 48 hours (n=4, p < 0.05).

Imazapyr had a detrimental effect on  $P_{M}^{B}$  only during chronic tests (Figure 3). Average  $P_{M}^{B}$  from three experiments remained relatively consistent (near 5  $\mu$ gC( $\mu$ gChl-a)<sup>-1</sup>hr<sup>-1</sup>) with all imazapyr additions during acute exposure experiments. The average  $P_{M}^{B}$  in control treatments was 5.48 ±0.61  $\mu$ gC ( $\mu$ gChl-a)<sup>-1</sup>hr<sup>-1</sup> (n=7). With +5 mg

L<sup>-1</sup>, +15mg L<sup>-1</sup> and +50 mg L<sup>-1</sup> imazapyr treatments  $P_{M}^{B}$  was 5.20 ± 1.07 μgC(μgChl-a)<sup>-1</sup>hr<sup>-1</sup> (n=7), 5.42 ± 1.29 μgC(μgChl-a)<sup>-1</sup>hr<sup>-1</sup> (n=4) and 5.05 ± 1.09 μgC(μgChl-a)<sup>-1</sup>hr<sup>-1</sup> (n=7) respectively. However, after 48 hours in the presence of the herbicide, there was a decline in the average assimilation number with increasing imazapyr concentrations. The mean control  $P_{M}^{B}$  during chronic exposure tests was 6.74 ± 0.33 μgC(μgChl-a)<sup>-1</sup>hr<sup>-1</sup> (n=7).  $P_{m}^{B}$  decreased with +5, +15, and +50 mg L<sup>-1</sup> imazapyr additions to 5.67 ± 1.34 μgC(μgChl-a)<sup>-1</sup>hr<sup>-1</sup> (n=7), 3.18 ± 1.78 μgC(μgChl-a)<sup>-1</sup>hr<sup>-1</sup> (n=4), and 1.33 ± 0.83 μgC(μgChl-a)<sup>-1</sup>hr<sup>-1</sup> (n=7), corresponding to a 16, 53 and 80% reduction respectively compared to the control.

As with the natural community, the experiment using cultured *Thalassiosira pseudonana* also showed a declining trend in  $P_{M}^{B}$  with increasing diuron concentration during the acute effect test (Figure 4).  $P_{M}^{B}$  in the control treatment was  $2.64 \pm 0.15 \, \mu g C (\mu g Chl-a)^{-1} h r^{-1}$  (n=3), but declined to  $1.97 \pm 0.28 \, \mu g C (\mu g Chl-a)^{-1} h r^{-1}$  (n=3) with an addition of +1  $\mu g \, L^{-1}$  diuron, to  $1.72 \pm 0.36 \, \mu g C (\mu g Chl-a)^{-1} h r^{-1}$  (n=3) with an addition of +2  $\mu g \, L^{-1}$  diuron and to  $0.94 \pm 0.05 \, \mu g C (\mu g Chl-a)^{-1} h r^{-1}$  (n=3) with +5  $\mu g \, L^{-1}$  diuron. This corresponds with a 25, 35 and 64% reduction respectively compared to the control.

Assimilation numbers remained fairly constant in the cultured diatom (T. pseudonana) experiment with increasing imazapyr concentrations during acute effect tests, but declined with imazapyr additions in chronic effect tests (Figure 5). There was a significant negative trend in  $P^B_M$  with increasing imazapyr concentrations for the chronic effect. After 48 hours, the control had  $P^B_M$  of  $1.37 \pm 0.19$  µgC(µgChl-a)<sup>-1</sup>hr<sup>-1</sup> (n=3). Between the +5 and +15 mg L<sup>-1</sup> imazapyr addition treatments the  $P^B_M$  decreased severely; with an apparent threshold concentration below which carbon assimilation did not occur. With a +15 mg L<sup>-1</sup> imazapyr treatment the  $P^B_M$  decreased to  $0.06 \pm 0.01$  (n=3; 96% decrease from the control) and with +50 mg L<sup>-1</sup> imazapyr,  $P^B_M$  was 0 (n=3; 100% decrease from the control).

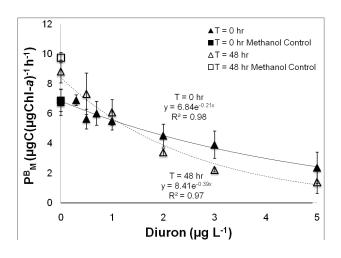


Figure 2 Average assimilation number (primary productivity normalized to chlorophyll-a biomass), PBM ± SD, versus diuron concentrations for 5 experiments conducted using water collected from central San Francisco Bay in 2009-10. Acute toxicity (dark triangles) is immediately after herbicide addition; the methanol control (dark square) is PBM with methanol only. Chronic toxicity (hollow triangles) is PBM measured 48 hours after herbicide additions; the methanol control (hollow square) is PBm in the treatment with methanol only. The solid trendline is for acute effects and the dashed trendline is for chronic effects. Acute n = 80, chronic n=37

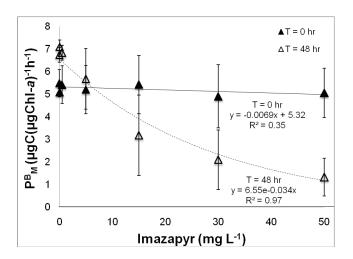


Figure 3 Assimilation number (primary productivity normalized to chlorophyll-a biomass), PBM  $\pm$  SD, versus imazapyr concentrations for 3 experiments conducted using water collected from central San Francisco Bay in 2010. Symbols and trendlines as in Figure 1 with no methanol control. n = 39

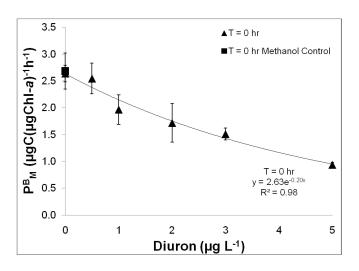


Figure 4 Assimilation number (primary productivity normalized to chlorophyll-a biomass), PBM ± SD, versus diuron concentrations using a monoculture of the diatom *Thalassiosira pseudonana*. Symbols and trendline as in Figure 1 for acute effects only. n=18

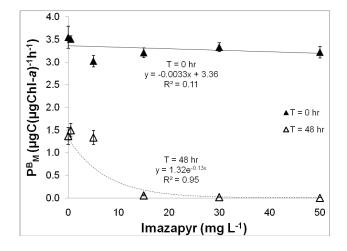


Figure 5 Assimilation number (primary productivity normalized to chlorophyll-a biomass), PBM ± SD, versus imazapyr concentrations using a monoculture of the diatom *Thalassiosira pseudonana*. Symbols and trendlines as in Figure 1 with no methanol control. n = 18

#### **Discussion**

Additions of both diuron and imazapyr significantly reduced phytoplankton productivity during experiments, although the concentration and exposure time required to elicit a response differed between herbicides. Results using a single species of cultured diatom in nutrient amended "clean" seawater medium corroborated the results for the mixed assemblage of phytoplankton collected in Central San Francisco Bay. The dose-response curve for diuron indicates that the herbicide had an inhibitory effect on phytoplankton photosynthesis at relatively low concentrations ( $\sim$ 1-2 ug  $L^{-1}$ ) and the effects were observed both immediately after exposure and with longer chronic exposure. In contrast, imazapyr impacts were observed with much higher additions ( $\sim 5 \text{ mg L}^{-1}$ ) than for diuron, and reduced assimilation number was only observed in more prolonged (48 hour) chronic exposure experiments.

The acute response of phytoplankton to diuron exposure is not surprising as the specific metabolic action of diuron is to block electron transport at Photosystem II, thereby preventing photosynthesis and oxygen production in photosynthetic organisms (Giacomazzi & Cochet, 2004). The results obtained here are consistent with other diuron exposure studies. The Environmental Protection Agency (EPA) sets water quality benchmarks for contaminants that receiving water can tolerate without adverse effects (Tenbrook et al 2009). The EPA aquatic benchmark for diuron for freshwater non-vascular plants is 2.4 μg L<sup>-1</sup> (EPA, 2010). Another contamination benchmark used to protect water bodies is the chronic criterion. Fojut et al. (2010) calculated a derived chronic criterion of 1.3 ug L<sup>-1</sup> for diuron based on a literature review of diuron toxicity levels on aquatic plants. Edmunds et al. (1999) found a lowest observable effect concentration of 2 ug L<sup>-1</sup> for diuron impact on phytoplankton. These concentrations that have a significant inhibitory effect on phytoplankton may occur at some times in the San Francisco Delta. Kuivila et al. (1999) described widespread occurrences of diuron (along with other herbicides) in the San Francisco-San Joaquin Delta with diuron concentrations reaching 2.14 µg L<sup>-1</sup> (Table 1). Johnson (2010) reports that 7.4 % of samples analyzed for diuron are expected to be above 2  $\mu$ g L<sup>-1</sup>.

The lack of an immediate effect of imazapyr on phytoplankton productivity likely reflects the fact that imazapyr does not directly affect photosynthetic reactions, but works by inhibiting the enzyme acetohydroxyacid synthase which catalyzes the production of 3 amino acids

important for protein synthesis and cell growth (Fisher et al., 2003). Because imazapyr does not interfere directly on photosynthetic pathways, but inhibits photosynthesis indirectly via protein synthesis, it likely requires longer exposure times to cause a decline in primary production, as was observed in the chronic experiments of this study. After 48 hours of exposure, imazapyr treatments of 15 mg L<sup>-1</sup> reduced primary production rates by half in the natural community and by 96% in cultured diatoms. The greater impact observed for the cultured diatoms suggests differences in sensitivity of individual phytoplankton species to imazapyr as noted by Pless (2005) and Brown (2010). This difference also may be related to differences in experimental conditions including using growth media with elevated nutrient concentrations and a higher initial biomass (chlorophyll-a) compared to the SFE experiments. Currently there are few scientific publications available on the effect of imazapyr on phytoplankton physiology and growth. Pless (2005) reported an  $EC_{50}$  for growth of 0.2 mg L<sup>-1</sup> for the freshwater green alga *Chlo*rella emersonii. The EPA reports an aquatic life benchmark of 11.5 mg L<sup>-1</sup> for non-vascular plants (EPA, 2010), similar to the range this study observed for toxic effects.

In 2006, the San Francisco Invasive Spartina Project reported the highest imazapyr concentration of 0.5 mg L<sup>-</sup> <sup>1</sup> for surface water adjacent to areas of herbicide application shortly after herbicide treatment (Kerr, 2007). However, imazapyr dissipated quickly due to dilution and degradation (Kerr, 2007). Based on these results, it seems unlikely for imazapyr concentrations to reach and maintain inhibitory levels for primary production in a mixed population given the longer time required to elicit an effect on productivity. However, there may be an effect on some sensitive species since herbicides are thought to play a role in phytoplankton community structure and inhibit more sensitive species, such as diatoms (Brown, 2009). An additional component of this study, not reported here, is to examine effects of these herbicides on community composition and may shed some light on this. There may also be places in the SFE with potential for imazapyr concentrations to be elevated due to less flushing and reach levels inhibitory to phytoplankton. For example, Ferner (unpublished data; Ferner, 2011, personal communication, see "Notes") measured imazapyr as high as 4.2 mg L<sup>-1</sup> in water at the edge of the treatment area following inundation of marsh plants treated with imazapyr.

With the growing concern for potential food limitation of pelagic fishes in the northern estuary (Mueller-Sol-

ger et al., 2002; Sommer et al., 2007), increased attention should be placed on understanding the potential role that herbicides may play in disrupting the pelagic foodweb. The limited published data for diuron concentrations in the San Francisco Estuary suggest that diuron concentrations are generally low, but at certain times or places diuron may be high enough to affect primary production. Imazapyr seems unlikely to have much of an impact on primary production in a mixed population, but may impact sensitive phytoplankton species.

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## Notes on the Morphology and Ecology of Non-native Hydrozoa Benthic Stages in the Brackish Waters of the San Francisco Estuary

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#### Introduction

A suite of Ponto-Caspian hydrozoan "jellyfish" have become established in the low salinity reaches of the San Francisco Estuary. Moerisia sp., Blackfordia virginica, Maeotias marginata and Cordylophora caspia likely arrived to this system via ballast water or hull fouling and may be competing with fishes for food resources (Wintzer et al. 2011). Hydrozoans possess a complex life history, including both pelagic medusae and benthic polyp stages, except for C. caspia, which is strictly a benthic organism. As is typical with jellyfish research, much of our knowledge of these species involves the conspicuous medusae phase, while the polyp stage remains understudied. In this article, we describe some key morphological features of the polyp phase that may aid with specimen identification and synthesize some of the existing ecological information published on these non-native fouling community members.

#### **Collection and Identification**

Fouling arrays were hung from May-November 2007 and April-November 2008 in the Napa and Petaluma Rivers, as well as Suisun, Montezuma, and Boynton sloughs in Suisun Marsh. Each array was suspended at two levels within the water column, one approximately 0.5m below the water's surface and the other 0.5m above the bottom. Six 100 cm<sup>2</sup> sheet PVC plates, roughed on both sides, were suspended from each level. Each plate was replaced monthly and preserved in 95% ethanol for further analysis (Wintzer et al. 2010).

Initial polyp identification was done with genetic sequencing, using the ITS1 primers method from Dawson and Jacobs (2001). These sequences were then compared to the ITS1 regions of medusae and a positively identified *C. caspia* polyp specimen. After genetic confirmation,

polyp species were classified based on morphology alone (Wintzer et al. 2010).

#### Morphology and Ecology

#### Moerisia sp.

Morphology

These solitary polyps take on both reproductive and feeding functions. They are attached to the benthos by a pedal disc that is covered in a chitinous sheath (perisarc). The main stalk is quite flexible and can stretch from 1mm to more than 10mm in length (Rees and Gershwin 2000). Tentacles extend in 1-2 rings from the polyp and have nematocysts at regular intervals, which give a beaded appearance. The feeding mouth (hypostome) is at the tip of a cylindrical proboscis at the top of the polyp (Mills and Sommer 1995). Medusa buds appear directly below the polyp tentacles and develop 4 tentacles of their own as they mature. These tentacles are often seen pulsing and tucked up inside the developing medusa (Wintzer, personal observation). Polyp buds (frustules) form slightly lower than the medusae on the polyp and resemble a simple finger-like projection. Occasionally, mature frustules may develop 1-2 tentacles (Mills and Sommer 1995). A single polyp can support many medusa buds and frustules, all in varying states of development (Rees and Gershwin 2000).(Figure 1)

#### **Ecology**

Moerisia sp. polyps were collected from the Napa (Mills and Rees 2000) and Petaluma Rivers, but they were in greatest abundance in Suisun Marsh (Wintzer et al. 2010). "Optimal" water conditions (based on data associated with the 5<sup>th</sup> and 95<sup>th</sup> percentiles of polyp recruitment) include 4.6-21.8% salinity, temperatures of 18.9-22.1°C, dissolved oxygen of 5.7-6.9 mg/L, and water transparency between 32.0-60.0cm (Wintzer et al. 2010). Polyps can enter a dormant stage in unfavorable water conditions (Mills and Sommer 1995). This species was collected in the field from floating docks (Mills and Rees 2000) and recruits to both vertical and horizontal structures, with greater numbers settling on the underside of horizontal surfaces compared to the upper-side (Wintzer et al. 2010). (Figure 2)

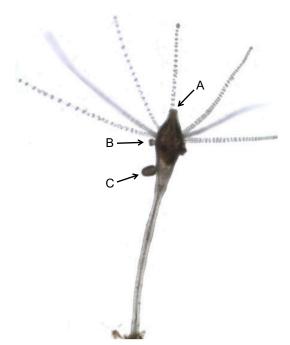


Figure 1 Polyp of *Moerisia* sp. A: hypostome, B: medusa bud, C: frustule.

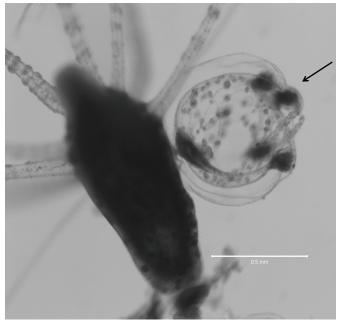


Figure 2 Close up of *Moerisia* sp. polyp with arrow indicating nearly-mature medusa.

#### Blackfordia virginica

Morphology

These polyps are colonial, with individuals (zooids) coming from tubular structures (stolons) that attach to the substrate (Bouillon et al. 2006). Polyps come in two forms which function in either reproduction (gonophores) or feeding (hydranths). Both types are delicate at >0.5mm in height (Mills and Rees 2000). Hydranths are protected by a chitinous capsule (hydrotheca), which is closed by an array of overlapping triangular flaps (operculum). The polyp can extend through the open operculum to capture prey. Tentacles are thread-like with little variation in thickness and are connected to each other at their bases by a membranous web (Bouillon and Boero 2000). Gonophores appear as rounded bulbous forms that may arise from the stolon or the stalk of a feeding polyp. A single medusa is found in each gonophore (Mills and Rees 2000). The colony can as exually produce more zooids through extensions of the stolon. (Figure 3)

#### Ecology

Blackfordia virginica polyps were found in great abundance in the Napa and Petaluma Rivers, with fewer in Suisun Marsh. The "optimal" water conditions for these polyps appear to include salinities of 14.9-22.2‰, temperatures of 20.0-23.1°C, dissolved oxygen of 2.3-6.3mg/L, and water transparency of 30.5-91.5cm (Wintzer et al. 2010). This species has been found on both floating docks and the barnacle Balanus improvisus (Mills and Rees 2000). Recruitment rates are greater for the underside of horizontal surfaces compared to the upperside (Wintzer et al. 2010).(Figure 4)

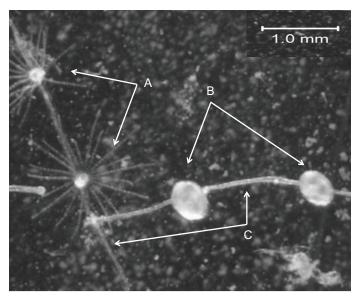


Figure 3 Colony of *Blackfordia virginica*. A: hydranths, B: gonophores, C: stolons.

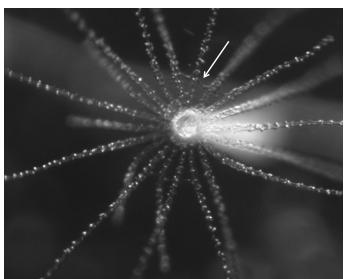


Figure 4 Close up of *Blackforsia virginica* polyp with arrow indicating inter-tentacle membrane.

## Maeotias marginata (Craspedacusta sowerbyi in lieu of M. marginata image)

Morphology

Information for the polyp phase of M. marginata is limited to the primary polyp stage, which is only 0.1mm in height and lacks tentacles. The morphology of this polyp is comparable to its better studied freshwater relative, Craspedacusta sowerbyi (Rees and Gershwin 2000), which is discussed below as a proxy. The morphology of C. sowerbyi includes both solitary and colonial polyps (composed of 2-3 polyps), each with a stocky base that tapers into a thinner neck region, followed by a crown (capitulum) of cnidocysts and the mouth. The polyp is flexible and can shrink and stretch from 0.5mm to 2mm in height. Craspedacusta sowerbyi is capable of 3 modes of asexual reproduction. New polyps may form as buds near the base and add to the colony, a non-colonial frustule may develop on the polyp stock, or a medusa bud may form (Acker and Muscat 1976; Bouillon et al. 2006). (Figure 5)

Ecology

Unknown

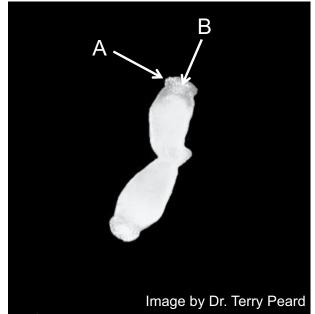


Figure 5 Two-polyp colony of *Craspedacusta sowerbyi*. A: capitulum of cnidocysts, B: mouth. This species is used to demonstrate the likely morphology of *Maeotias marginata* polyps.

#### Cordylophora caspia

Morphology

Polyps are colonial and have both gonophores and feeding hydranths. The branched colonies are comprised of stolons, from which main stems (hydrocauli) arise. Each hydrocaulus can then support multiple secondary branches (pedicles). Colonies can reach several centimeters in height are covered in protective perisarc, except for the hydranths. Hydranths, located at the end of each pedicle, possess thread-like tentacles with evenly distributed cnidocysts. The tentacles are found over much of the polyp head, and a conical hypostome is at the top of the polyp (Bouillion et al. 2006). Colonies are dioecious, with their gonophores producing either sperm or eggs (Folino 2000). Gonophores are bulbous, oval-shaped structures that arise on the pedicles below the hydranths (Bouillion et al. 2006). Fertilization from gonophores creates free-swimming planulae larvae, which settle on the benthos to form new colonies. Additionally, C. caspia asexually produces more polyps within its colony (Folino 2000). (Figure 6)

#### Ecology

This species is found in the Napa and Petaluma Rivers (Schable et al. 2008), as well as Suisun Marsh (Wintzer et al. 2010). Its "optimal" conditions include 1.4-8.9‰ salinity, temperatures of 18.1-23.5°C, dissolved oxygen range of 4.6-7.6mg/L, and water transparency of 12.0-55.0cm (Wintzer et al. 2010). Colonies enter a state of dormancy when water conditions are not favorable. *Cordylophora caspia* can settle on a variety of surfaces, including intake screens (Folino-Rorem and Indelicato 2005), rocks, bivalve shells, and vegetation (Roos 1979). It has even been found growing on the dorsal surface of a live Sacramento splittail (A. Wintzer, unpublished observation)! This species recruits to both vertical and horizontal surfaces (Wintzer et al. 2010).

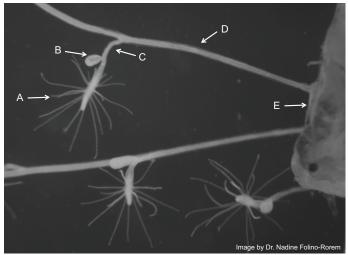


Figure 6 Colony of *Cordylophora caspia*. A: hydranth, B: gonophore, C: pedicle, D: hydrocaulus, E: stolon.

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### **Mysid Abundance in a Restored Tidal Freshwater Wetland**

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#### Introduction

The mysid is a small crustacean that serves as a key prey item to many pelagic fish species of the Sacramento-San Joaquin Bay-Delta Estuary (Estuary) that are now declining, such as the juvenile striped bass (*Morone saxatilis*) and white and green juvenile sturgeon (*Acipenser transmontanus* and *medirostris*, respectively), longfin smelt (*Spirinchus thaleichthys*), and the American shad (*Alosa sapidissima*) (Heubach et al. 1963; Steven 1966, Radtke 1966; Bryant and Arnold 2007). Mysids were once an abundant and preferred prey item able to support these fish populations, particularly as juveniles, throughout the Sacramento-San Joaquin Bay-Delta Estuary.

Reductions in mysid abundance caused many pelagic fish species to shift their diet focus to other available prey, which may be energetically inferior to the once abundant mysid food source (Nobriga & Feyrer 2008; Kimmerer 2000). In addition to overall abundance declines, a shift in mysid composition within the Estuary has occurred: the native *Neomysis mercedis* was historically the most abundant mysid, now *Hyperacanthomysis longirostris*, introduced in the early 1990's, is the most abundant mysid in the Estuary (Winder and Jassby 2010).

Mysid abundance in the Estuary is often concentrated in tidal marshes (Dean et al. 2005). Tidal freshwater wetlands, such as Liberty Island in the Cache Slough Complex, are increasingly recognized as valuable habitat in the Sacramento-San Joaquin Delta due to their potential to provide enhanced food web productivity to neighboring pelagic habitats (Brown 2003). Chlorophyll concentration, an indicator of phytoplankton primary productivity, has been consistently low throughout the Delta since the late 1980's due to the establishment of filter feeding bivalves Corbula amurensis and Corbicula fluminea (Jassby et al. 2002, Lucas et al. 2002). The effects of this major change in the food web are believed to have cascaded up the pelagic food web, causing declines in abundance of zooplankton, mysid shrimp, and fish (Feyrer et al. 2003). As a result, there have been management directives calling for the restoration of tidal freshwater wetlands in the delta to benefit threatened and endangered fish populations, particularly Chinook salmon (Oncorhynchus tshawytscha) and delta smelt (USFWS 2009; NMFS 2009).

The Cache Slough Complex is one of the primary regions targeted for future habitat restoration (USFWS 2009; NMFS 2009; BDCP 2010). Preliminary results from intensive physical and biological sampling in this region confirm habitat benefits of the Cache Slough Complex. For example, sediment sampling conducted by Morgan and Schoellhamer (in review) from USGS found the Cache Slough region to be more turbid than the channelized freshwater Delta. Turbidity in this region provides native pelagic fish with refuge from predation and enhances feeding efficiency (Feyrer et al. 2010). Biological sampling by DWR recently found chlorophyll concentration in this region exceeds the threshold of food web limitation (>10 µg L<sup>-1</sup>, Müller-Solger et al. 2002); as a result, calanoid copepods, an important food source for pelagic fish, are more abundant in the Cache Slough Complex compared to some other parts of the Delta (Benigno et al. in preparation).

Here, we present preliminary results on the temporal and spatial patterns of mysid abundance and species composition in the Cache Slough Complex in order to further our investigation of the potential Cache Slough Complex "food bank" for the Estuary. We focus on mysids in these preliminary results because of their importance as a food resource for pelagic fishes. Specifically, we investigated 1) diel and seasonal patterns in mysid abundance, 2) relative abundance of both *N. mercedis* and *H. longirostris* in three regions of the Cache Slough Complex, and 3) the size distribution of each mysid species over time and between Cache Slough Complex regions.

#### Methods

#### **Study Site**

The Cache Slough Complex, an entirely freshwater region, is comprised of a network of shallow dead-end sloughs, the Sacramento Deep Water Ship Channel, and freshwater tidal wetlands in the Northern portion of the Estuary. Three sites were sampled in the region: Liberty Island (LI), the Sacramento Deep Water Ship Channel (DC), and Cache Slough (CS) (Figure 1). The largest hydrological feature is the former agricultural island Liberty Island, located at the southern end of the Yolo Bypass. The island became permanently inundated when the levees failed in 1997. This passive restoration created 5,000 acres of subtidal and tidal marsh habitat. Sample site LI is located at the base of Liberty Island at a major water entry way that connects the island with Cache Slough. Another prominent feature is the Sacramento Deep Water Ship Channel, constructed in 1963, which has developed extensive shallow shoals and emergent wetland vegetation along its channel margins over the last 50 years. Sampling site DC was located near the southern end of the Deep Water Ship Channel, a deep man made corridor that extends beyond Cache Slough. Cache Slough connects the Cache Slough Complex with the Sacramento River just upstream of Rio Vista. The sampling site within Cache Slough, CS, for this study was located midway between Liberty Island and Rio Vista, well within the reach of tidal exchange from Liberty Island.

#### **Data Collection**

Eight 24 hour sampling events were conducted quarterly from June 2008 to April 2009 at these sties over two consecutive weeks each season to capture spring and neap tidal cycles. A 30cm diameter, 500 micron mesh net was used to conduct 10 minute oblique tows every 1.5 hours to

sample from the bottom to the surface of the water column. General Oceanics mechanical flow meters were fitted in the mouth of each net to record the volume of water sampled. Samples were preserved in 5% buffered formalin with Rose Bengal dye.

For the subset of data used in this paper, the two samples collected closest to the time of daytime and nighttime high slack tides were analyzed. A total of 94 samples were processed using a dissecting microscope. Mysids were counted, identified to species, and categorized as juvenile, under 2 mm, adult, or as a gravid female (if a visible yolk sac was present). Individuals less than 1 mm were not included due to the inefficiency of mesh size used to efficiently sample smaller life stages. High slack tide samples were used in order to be comparable with the Interagency Ecological Program's Environmental Monitoring Program (EMP) long-term monitoring stations that span Suisun Marsh and the Delta, which are also sampled at high slack tide. Up to 100 individuals per sample were randomly measured to standard length by caliper to the nearest mm. Data from each sample was converted to density (number of organisms per cubic meter) based on the volume of water sampled from flow meter readings.

#### **Data Analysis**

Mysid abundance (expressed as number of organisms per cubic meter of water  $\pm$  standard error) was analyzed to evaluate diel, seasonal and sample location differences. Diel patterns were analyzed with a General Linear Model (GLM) using Minitab-13 data analysis software. The occurrence of life stages (juvenile, adults, or gravid female) across these variables was evaluated geographically. Frequency of occurrence was used to understand mysid size distribution across samples sites and seasons. Mysids from 1-14 mm were categorized into size classes of 2mm. The category label indicated the maximum length included in the size class (Size class 2 = 1.0 - 2.0mm; size class 4 = 2.1 - 4.0mm; etc.).

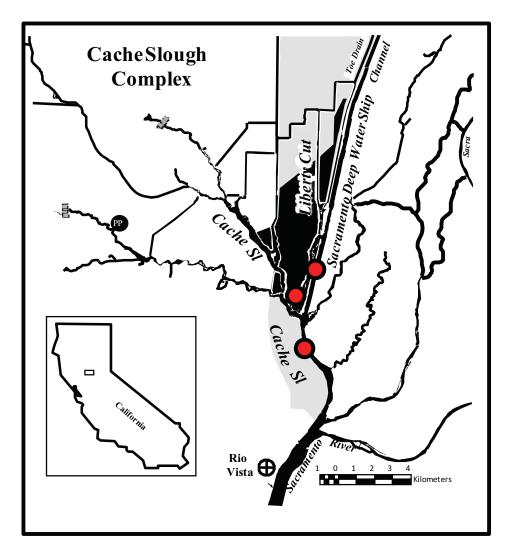


Figure 1 Map of sites sampled in the Cache Slough Complex from summer 2008 to spring 2009.

#### Results

From the 94 samples that were processed, a total of 1,773 mysids were identified. Sample density ranged from zero to 10.01/m<sup>3</sup>. Both introduced (*H. longirostris*) and native (*N. mercedis*) species were collected in this region.

Average mysid abundance was greater at night (GLM, p=0.005). Average day abundance was  $0.08/m^3 \pm 0.036$  and average night abundance was  $1.00/m^3 \pm 0.360$ . This trend was seen across all seasons and sites (Figure 2). Mysids were found during the winter only in nighttime samples.

Diel patterns of gravid female abundance are of particular interest (Figure 2). Daytime gravid female mysids

were only present at low densities in 1 out of 12 samples collected during the summer  $(0.002/m^3 \pm 0.002)$ , and absent from daytime samples in all other seasons. In contrast, nighttime samples contained gravid throughout the year.

Seasonal abundance varied greatly between summer, when abundance was highest, and other seasons (Figure 3). Mysid abundance was not significantly different between spring and neap tidal cycles (GLM, p=0.930). Differences between samples sites was significant (p= 0.010), with DC and LI having similar mysid abundance patterns while CS had greater abundances, particularly in the summer  $(4.6/m3 \pm 1.69)$ .

Hyperacanthomysis longirostris was the most abundant mysid collected (Figure 3). Annual average abun-

dance for H. longirostris was  $0.490/m3 \pm 0.187$  and N. mercedis was  $0.030/m3 \pm 0.007$ . However, there was a seasonal shift in abundance that allowed the two species to dominate different periods of the year.  $Hyperacantho-mysis\ longirostris$  accounted for 97% of the summer and 90% of the autumn mysids collected. After autumn, N. mercedis became dominant and was the only mysid present during winter and spring.

For all sample sites, mysids ranging from 2-6 mm (Size classes 4 and 6) were the most abundanct of all size classes (Figure 4). The largest mysids, size classes 12 and 14, were present in site CS (size class 12 only) and LI. Percent species composition indicated species ratios for each size class (Figure 5). *Hyperacanthomysis longirostris* made up at least 80% of size classes 4, 6 and 8. Mysids in size classes 10, 12, and 14 were 100% *N. mercedis*. Large mysids from size class 12 and 14 were caught during winter (LI only) and spring (LI and CS) (Figure 6). These large mysids were absent from all sites in the summer and autumn, and were never sampled from DC throughout the year.

#### **Discussion**

The purpose of the study was to begin to investigate temporal and spatial patterns of the mysid community within the Cache Slough Complex, as a step toward the broad goal of understanding the mesozooplankton contribution to a potential food web within a freshwater tidal wetland in the Estuary. Three key points from this study provide insight into the mysid community and availability as a food source within the region: 1) Diel differences in abundance demonstrate the limits of our understanding of the mysid community due to sampling technique; 2) seasonal variation in relative abundance of the two mysid species revealed alternate periods of peak abundance for N. mercedis and H. longirostris; and 3) mysid size distributions illustrated differences between species in both their maximum size and the seasonal timing for maximum size. In particular, interesting trends were observed for gravid female mysids with regard to diel patterns, seasonal variation, and size differences. Because our sampling was limited to the seasons of one year (Summer 2008-Spring 2009) our results are limited in their ability to evaluate long-term trends in mysid abundance within the region and in comparison with the rest of the freshwater Delta.

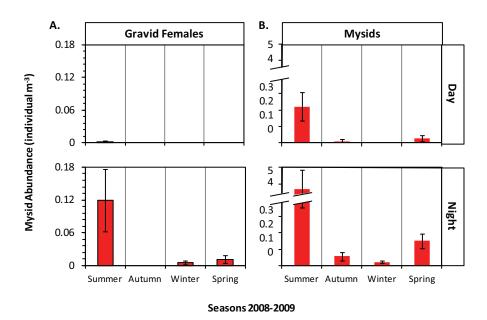


Figure 2 Average daytime and nighttime seasonal mysid abundance from summer 2008 to spring 2009. (A) Gravid female abundance. (B) Total mysid abundance.

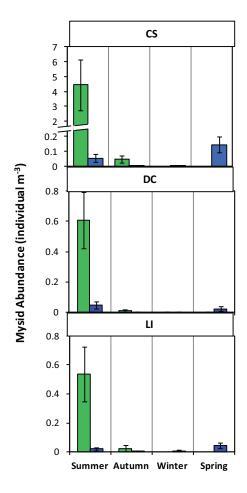


Figure 3 Comparison of total mysid abundance between sampling sites CS, DC, and Ll. *H. longirostris* is shown in green and *N. mercedis* is shown in blue.

Nighttime mysid abundance was higher than daytime abundance. Mysids migrate vertically; they form planar schools in bottom waters during the day and spread out into shallower water swarms at night (Whittman 1977; Ritz 1994; Kringer et al. 2003; Abello et al. 2005; Ohtsuka et al. 1995). Variation in abundance could be due to multiple factors. Fast schooling daytime mysids may be able to efficiently transfer information to each other to avoid nets (Clutter & Anraku 1968). Secondly, it is important to note that oblique tows may not efficiently sample the bottom where mysids could be residing during the day (Whittman 1977; Jumars 2007). More effective sampling methods of the bottom would yield a more effective description of mysid abundance. By sampling at night we were able to provide a more detailed description of the mysid community, including higher overall abundance and the presence of seasonal gravid females in winter and spring.

Seasonal variation between the 2 species within the Cache Slough Complex was consistent with other regions of the freshwater Delta. Environmental Monitoring Program long-term monitoring stations located upstream of the low salinity zone represent the freshwater Delta, but the Cache Slough Complex is not included in the EMP sampling areas. In this study, we have shown that in this region, H. longirostris abundance is greater than N. mer*cedis*; however, *H. longirostris* is least abundant while *N*. mercedis is most abundant in the upstream low salinity zone compared to the regions at and below the low salinity zone. Environmental Monitoring Program sampling results in 2008 for the upstream low salinity zone were consistent with previous monitoring years although overall abundance was low. Specifically, H. longirostris abundance peaked during the summer (May through July) and fell by November. N. mercedis had low abundance in spring, a peak during June and then were not detected in samples collected after July. Although we sampled quarterly rather than monthly, our results for the Cache Slough Complex were consistent with EMP data, with the highest abundance of *H. longirostris* in summer and fall. In our data set *N. mercedis* was most abundant in Spring and was present throughout the year.

Average mysid size was consistent with the average mysid size of the freshwater Delta. Winder and Jassby (2010) analyzed average annual mysid size from the 1970s to 2008 and reported a steady decline throughout the Sacramento-San Joaquin Bay-Delta Estuary, the average reaching 4.4 mm for the 2000s. Similar to the rest of the freshwater Delta, H. longirostris is more abundant but smaller than N. mercedis (Orsi 1997). In our data set H. longirostris did not exceed 7.5 mm in length, and N. mercedis ranged up to 13 mm, and the overall average annual length for both species reaching 4.3 mm (Figure 4). High abundance and competition for food by *H. longirostris* may be contributing to the declining average mysid size (Orsi & Mecum 1996). Furthermore, H. longirostris reproduces at smaller life stages, carries more eggs compared to *N. mercedis* of equivalent length, and contains potential for its smaller eggs to develop faster than those of N. mercedis (Orsi 1997). These characteristics and its greater abundance allow *H. longirostris* to outcompete *N*. *mercedis* when it is present in the system, and could be contributing to the smaller sizes seen in N. mercedis due to food limitation (Orsi 1997; Siegfried 1979). However, N. mercedis reached up to 14 mm during the spring and winter, when *H. longirostris* is absent from the region. During the winter and spring, *N. mercedis* is able to

develop to an overall larger size and is more fecund compared to *N. mercedis* maturing during the summer (Siegfried 1979; Orsi & Mecum 1996). This difference in timing of peak abundance may reduce food competition between *N. mercedis* and *H. longirostris*, and allows the young of *N. mercedis* during the winter to have a chance to feed before the abundance of *H. longirostris* increases in the system.

These data comprise a subset of a larger mesozooplankton sampling effort that will further analyze mysids and other water column invertebrates within the Cache Slough Complex. The overall purpose of the mesozooplankton study is to better understand how mysids and other mesozooplankton support Delta food webs. In these preliminary results, we found that the Cache Slough Complex contains mysids year-round that support pelagic fish populations, particularly in the winter when the rest of the freshwater Delta is lacking this important food item. Furthermore, the most abundant seasons for the native N. mercedis are winter and spring, when competition for food is lowest and average size and fecundity is highest. In particular, mysids were present year round, with the highest abundance during summer and fall, and the largest mysids in winter and spring. This region provides pelagic fish with an important targeted previtem. Our results provide additional evidence of enhanced food web resources in the Cache Slough Complex. Our hope is that this information will help inform the design of habitat restoration projects in the Delta.

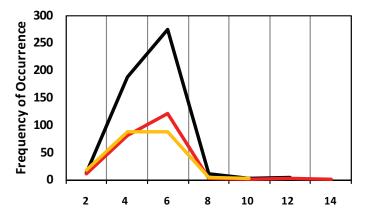


Figure 4 Size Class frequency of occurrence of mysids caught from Summer 2008 to Spring 2009.

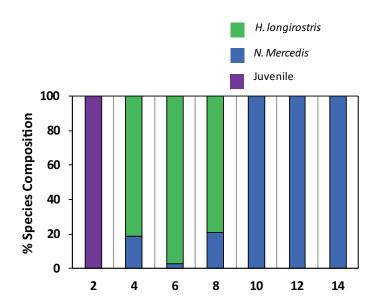


Figure 5 Size class percent species composition of mysids caught.

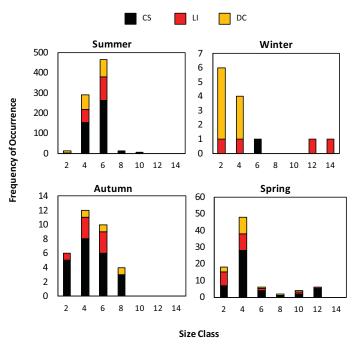


Figure 6 Size class frequency of occurrence of total mysids caught by season.

#### **Acknowledgements**

We would like to acknowledge the Cache Slough Study Principal Investigator Ted Sommer and the IEP for funding. Thank you to our DWR field crew; Megan Anselmo, Nick Van Ark, Duncan Sommer, and Jocelyn LaBelle for assisting in sample identification and sorting; Kathy Heib and April Hennessey from the California Department of Fish and Game for advising us on sampling protocol and mysid identification.

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# Spawning of Wakasagi *Hypomesus* nipponensis at Los Vaqueros Reservoir

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Eggs from the introduced osmerid wakasagi were collected from Los Vaqueros Reservoir on February 11, 2010. Eggs were collected from the northeast cove of the reservoir where the Adobe Creek, an intermittent stream, empties. Temperature at the collection site was 11.2 °C, dissolved oxygen level of 10 mg/L, and salinity of 0.2 ppt. There was light precipitation the day before collection and the water was slightly turbid. Eggs were collected from shallow water (15-45 cm deep) with little to no water movement, and were attached to submerged, horizontallypositioned dead vegetation. Other substrates available were silt and mud; however, as expected, no eggs were observed since silt and mud are not good substrate for egg attachment. Egg concentrations were heaviest in vegetation that was decomposing into thin threadlike strands of fiber. Eggs were translucent, had a diameter between 0.85–1.0 mm, and had an adhesive anchor made from the chorion, a characteristic found in osmerids (Wang 1986). The eggs were of different embryonic stages even within the same strand of fiber substrate, ranging from newly fertilized to advanced eyed embryo. The newly fertilized eggs were in the high blastomere stage meaning that the eggs were probably only a few hours old. The advanced eyed embryos were likely several days or weeks old (incubation period for wakasagi in the laboratory can reach 3 weeks at 14 °C). This age diversity means that there were several spawning events before our collection. To verify the species, the eggs were incubated and the larvae raised to juvenile stage.

These naturally spawned eggs collected from an adjacent reservoir of the Sacramento-San Joaquin Delta may be the first documented wakasagi egg collection from the system. Wakasagi eggs were collected from the Portuguese Cove in San Luis Reservoir (J. Wang, personal communication 2010) by Hess et al. (1995); however, only collection of wakasagi prejuveniles and juveniles were mentioned by Hess et al. Locating osmerid eggs and spawning microhabitat in the system, especially for delta smelt *Hypomesus transpacificus*, is difficult. Since wakasagi and delta smelt share several ecological traits,

spawning information of wakasagi may provide clues to finding delta smelt eggs.

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# **Using Harvest Rate and Harvest to Estimate White Sturgeon Abundance**

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#### Introduction

The California Department of Fish and Game (CDFG) has estimated abundance of white sturgeon (Acipenser transmontanus) in the San Francisco Estuary many times for several decades using a complicated algorithm. The algorithm (and application thereof) includes (1) periodic updates with recapture data collected up to several years after tagging, (2) assumptions about growth rate and about mortality attributable to tagging, and (3) more professional judgment than we'd like. Aside from their infrequent use when considering regulation of the fishery and the impact of development, abundance estimates are used each year to monitor progress toward the CVPIA 'Doubling Goal' for both white sturgeon and green sturgeon. Because the estimates are imprecise and take years to develop, their use in any near-real time sense is very limited. Here we describe and briefly explore an alternative method of estimating white sturgeon abundance that is precise and can be finalized relatively quickly. The alternative method uses estimates of harvest rate and uses harvest data from Sturgeon Fishing Report Cards.

#### **Methods and Results**

#### Abundance Estimates

We estimated the abundance of white sturgeon 117-168 centimeters total length (cm TL) by dividing harvest by harvest rate (Table 1). The size range is dictated by — and identical to — the legal limits on harvest of white sturgeon in California since March 2007.

Anglers are required to document the date and location of harvested fish on Sturgeon Fishing Report Cards (Cards) and are required to submit Cards by January 31 of the following year. Harvest is simply the number of fish that anglers reported harvesting.

Harvest rates are estimated by dividing the number of tags returned (by anglers) by the number of tagged fish released by the CDFG (DuBois 2011a) and can be (but was not in this instance) adjusted to address factors that may bias the estimate (e.g., tagging-induced mortality). Because the CDFG releases tagged fish only during August-October, harvest rate estimates — though reported per calendar year — are actually for the period of August-October in Year-X to August-October in Year-X+1.

To assure that the estimates of abundance are calculated using values for harvest and harvest rate that are reasonably synoptic and as an exploratory analysis, we considered harvest for 3 periods: (1) 365 days from beginning of tagging (1-beg); (2) 365 days from midpoint of tagging (2-mid); and (3) 365 days from the end of tagging

(3-end). The period over which harvest was summarized made little difference in the estimate (Table 1).

#### **Confidence Intervals**

Asymptotic normally-distributed (Wald-type) upper and lower 95% confidence intervals (CI) were estimated per methodology developed by Ken Newman (pers. comm.). This type of interval assumes a normal distribution of the data (i.e., abundance estimates in this case) and was calculated using the equations below, where  $SE(\hat{A}) =$  standard error of the abundance estimate. Lower and upper confidence intervals (at 95%) were calculated as  $\hat{A} \pm CI$ .

$$SE(\hat{A}) = \frac{Harvest}{\sqrt{Harvest\,Rate^3 \times Number\ of\ tags\ released}}$$
 
$$CI = 1.96 \times SE(\hat{A})$$

Despite a skewed distribution of abundance estimates simulated via Poisson distribution (N=5,000) using 2007 1-beg data (Figure 1), the Wald-type intervals provide good coverage of the abundance estimate (Figure 2). Poisson distribution simulations (N=5,000) using 2008 and 2009 data produce similar distributions and thus yield the same conclusion.

Table 1 Estimated abundance of white sturgeon 117-168 cm TL using harvest and harvest rate (see DuBois 2011a for harvest rate estimates).

Year	Estimate Period	Period From	Period To	Harvest	Tags Released	Tags Returned	Harvest Rate	Estimated Abundance	Lower 95% CL	Upper 95% CL
2007	1-beg	08/03/07	08/01/08	1,931	388	13	0.034	56,794	26,146	87,442
	2-mid	09/14/07	09/12/08	1,918				56,412	25,970	86,854
	3-end	10/25/07	10/23/08	1,829				53,794	24,765	82,823
2008	1-beg	08/11/08	08/10/09	1,902	320	14	0.044	43,227	20,648	65,807
	2-mid	09/20/08	09/19/09	1,914				43,500	20,778	66,222
	3-end	10/29/08	10/28/09	1,931				43,886	20,963	66,810
2009	1-beg	08/10/09	08/09/10	1,397	286	9	0.031	45,065	15,401	74,728
	2-mid	09/18/09	09/17/10	1,397				45,065	15,401	74,728
	3-end	10/27/09	10/26/10	1,361				43,903	15,004	72,803

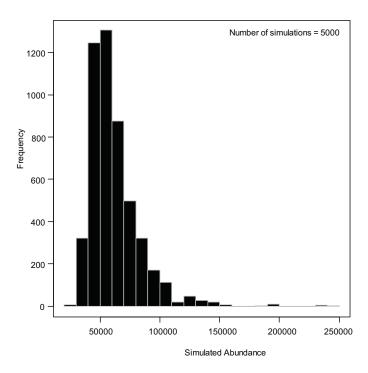


Figure 1 The distribution of simulated estimates of white sturgeon abundance (117-168 cm TL) in 2007 using harvest and harvest rate

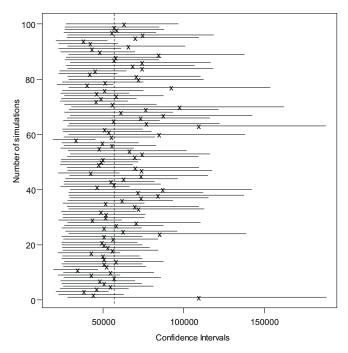


Figure 2 Confidence intervals for simulated estimates of white sturgeon abundance (117-168 cm TL) in 2007 using harvest and harvest rate. X = simulated abundance estimates (N=100); dashed vertical line = 2007 abundance estimate (56,794)

#### **Discussion**

The degree to which estimates made using the alternative algorithm have management utility depends in large part on their cost, timeliness, and precision. The annual cost (excluding postage paid by anglers) of Sturgeon Fishing Report Cards data has been approximately \$25,000 and should decrease with full implementation of the Automated License Data System. Estimates using the alternative algorithm can be finalized within about a year, which is several years sooner than estimates have been finalized using the conventional algorithm. Because estimates made using the alternative algorithm do not require updating, their precision — unlike the precision for estimates made using the conventional algorithm (Miller 1972; DuBois 2011b) — is not an issue.

Cost, timeliness, and precision of these abundance estimates are moot if accuracy (trend-wise and/or absolute) of the estimates is not good enough. Accuracy is notoriously hard to evaluate and is beyond the scope of this article, but we will approach it here through a brief exploration of biases for the alternative algorithm and a brief review of estimates made using both algorithms.

Accuracy of estimates using the alternative algorithm is impacted by the net effect of several likely biases. Because none of those biases have been quantified recently (if ever), the following speaks mostly to their likely directions and suggests that the biases tend to offset:

- (1) If harvest rate is underestimated, then estimates made using the alternative algorithm are biased high. We believe harvest rate is likely under-estimated due to under-reporting by anglers, mortality attributable to tagging, and tag shedding (Ricker 1975). With new research (e.g., a double-tagging study), additional outreach (e.g., posters alerting anglers about tagged sturgeon), and the inclusion of a tagged-fish section of 2010 and later Cards, we hope to reduce and quantify the impact of these issues.
- (2) If harvest is underestimated, then estimates made using the alternative algorithm are biased low. While both under- and over-reporting of catch by anglers is possible, we have heard from anglers and law enforcement that under-reporting is the more-common of the two. We are in the "shall-we-do-this" stage of planning a study with law enforcement to quantify the degree of under-reporting.

Abundance estimates from the two algorithms vary no more than about  $\pm$  5,000 for 2008 and 2009 and no more than about  $\pm$  20,000 for 2007, suggesting that the alternative and routine algorithms generally track the

same trends in abundance. Although several more estimates made using both approaches will be required before we can reasonably describe their statistical relationship (e.g., through regression), these initial signs of accuracy are promising.

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#### **Notes**

Ken Newman (Mathematical Statistician, US Fish and Wildlife Service), e-mail, 27-Jan-2011

## Length-at-Date Criteria to Classify Juvenile Chinook Salmon in the California Central Valley: Development and Implementation History

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#### Introduction

California is unique in having four different spawning runs of Chinook salmon in the Sacramento River, resulting in a mixed population of juveniles in the river and downstream habitat. Identifying the offspring of these four runs (fall, late-fall, winter and spring) is particularly challenging as the runs are distinguished by the timing of adult spawning migrations, rather than juvenile behavior or appearance. The current solution is to classify the run origin of juveniles in this mixed population using lengthat-date size criteria. Length-at-date criteria are the expected fork-length ranges of each run at each calendar date. Length-at-date criteria are organized into tables such that the fork-length of any Chinook salmon juvenile encountered in the Central Valley can be compared to the expected length ranges for the encounter date, and classified to run accordingly. Length-at-date classification is the accepted approach for designating run origin of juvenile Chinook salmon in the Sacramento and San Joaquin rivers and the Delta, and is central to loss and take estimates of threatened and endangered Chinook salmon runs at state and federal water pumping facilities. Since take estimations can affect the operations of the California State Water Project (SWP) and the federal Central Valley Project (CVP), the accuracy or inaccuracy of run classifications has enormous implications both for the persistence of Chinook salmon runs and for water use in California. Considering the importance of salmon and water to the California economy, it is surprising that the development of length-at-date size criteria is so poorly documented that few people are aware of the theory, assumptions and supporting data upon which the criteria are based. Following is an account of the development and implementation history of length-at-date size criteria for juvenile Chinook salmon in the California Central Valley. As the details of this account were pieced together from memoranda, meeting minutes and unpublished draft reports, those who par-

ticipated in this history may find inaccuracies with their own recollections. Corrections may be directed to the author for inclusion in future revisions of this document.

#### **Regulatory Basis for Run Classification**

In February 1992, as a result of the listing of Sacramento River winter-run Chinook salmon as threatened under the federal Endangered Species Act, National Marine Fisheries Service (NMFS) issued a biological opinion including an incidental take permit for operations of the SWP and the CVP in the Sacramento-San Joaquin Delta {NMFS, 1992 #37}. The juvenile winter-run take limit was set at 1% of each year's estimated population. Since juvenile Chinook salmon in the Delta was a mixed population of progeny from the four Central Valley runs, a method for designating run-origin for juvenile salmonids was needed to tally the take of winter-run versus nonwinter run Chinook salmon at the SWP/CVP water pumping facilities.

## Initial Efforts to Develop Length-at-Date Criteria

A length-at-date approach for identifying winter-run Chinook salmon juveniles was originally proposed by Stevens (1989) to estimate the timing of winter-run outmigration through the Delta. Stevens, a fisheries management supervisor at the Department of Fish and Game (DFG), observed that adult Chinook salmon within a given run tend to spawn, and their progeny emerge, at the same general time of each year, while the spawning and emergence times of different runs tend to be segregated. From this he reasoned that the range of juvenile forklength for any given calendar date could be estimated by determining the earliest and latest emergence times of each run and then extrapolating size at emergence into the future by applying knowledge of juvenile growth rates. Stevens enlisted Frank Fisher (DFG) to plot points on a two-dimensional graph with the earliest and latest emergence time of each run as the x-coordinate, and the average size of Chinook salmon at emergence as the ycoordinate. Each point served as an intercept for a separate log-linear regression line, with the slope of all lines equal to a Chinook salmon growth-rate estimate used by hatchery managers at the time (Figure 1).

Fisher (1992) recognized that the hatchery-based growth rate, which assumed a doubling of fish weight

every month, over-estimated growth of naturally occurring salmon, and was therefore inadequate for estimating wild Chinook salmon length-at-date. However, Fisher also recognized that growth rate estimation in wild Chinook salmon populations was complicated by variability in emergence times, sampling inefficiencies, immigration and emigration. As a compromise, Fisher opted to replace the growth rate from Stevens (1989) with a growth rate estimated from juvenile fall-run Chinook salmon that had been spawned and reared in artificial spawning channels attached to the Tehama-Colusa Canal near Red Bluff Diversion Dam. Fisher described the growth rate of these juveniles as "natural" because the juveniles were produced by natural spawning activity of ripe adults placed in the spawning channel and the embryos were incubated in gravel. However, "natural" is a somewhat inaccurate descriptor since juveniles in the Tehama-Colusa Canal were reared on hatchery food, and juvenile densities were artificially maintained in the spawning channels.

To estimate growth rate, Fisher (1992) calculated average condition factor (CF) from weight and count data of Chinook salmon juveniles, compiled at weekly intervals from 1972-1981, where

$$CF=0.000730-0.00005*In(count/weight)$$
 (1)

The parameters of this equation were derived from eleven measurements of average CF that had been taken at different stages of juvenile growth and then regressed against fish count per bulk fish weight. The report does not document the origin of these eleven data points. The standard equation for condition factor,

$$CF = 10^5 * mass/(fork-length)^3$$
 (2)

was manipulated to convert average CF for each week to average fork-length,

fork-length = 
$$(10^5 * mass/CF)^{1/3}$$
 (3)

Note that average mass per fish was estimated by dividing the weight of a fish sample by the count of fish in the sample (weight/count), while average CF was estimated from the reciprocal of this quotient (count/weight) using equation 1. Average fork-length data derived from equation 3 were pooled to estimate the parameters for a log-linear, fork-length growth equation. Since the above equation yielded an emergence size at day zero (31 mm)

that was smaller than the average observed Chinook salmon emergence size (34 mm), Fisher adjusted the intercept of the equation to force a 34 mm fork-length at emergence (day zero) while maintaining the same growth rate, resulting in,

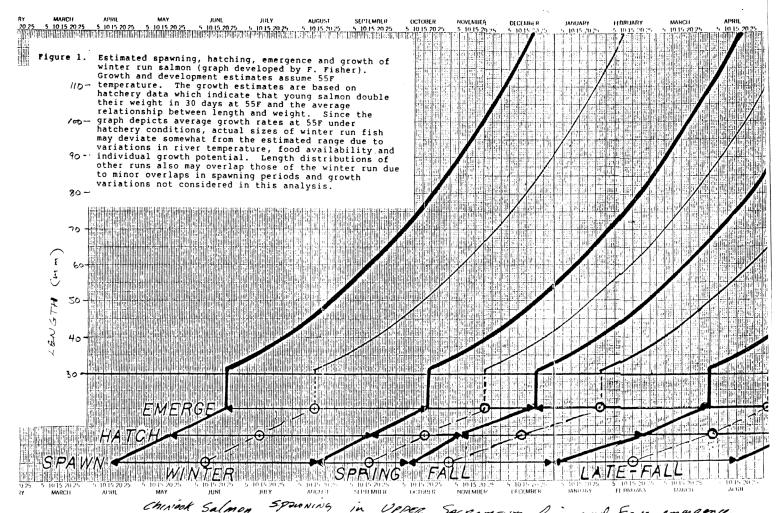
$$ln(fork-length) = 3.516464+0.006574 * days (4)$$

where days is the time from peak emergence of fry in the Tehama-Colusa Canal spawning channel, assessed separately for each year.

Substituting early and late emergence times of each Chinook salmon run for days in equation 4, Fisher used this fork-length growth-rate equation (based on fall-run Chinook salmon) to construct a table of expected forklength ranges for juveniles of all four runs in the Central Valley (Figure 2). Early and late emergence times for each run were estimated as the number of days required for eggs to accumulate 1500 temperature units following the date of early and late spawning activity where average early and late spawning activity for each run was based on Hallock (Hallock 1973) and other reports of spawn timing. A temperature unit is accumulated for each degree Fahrenheit exposure above freezing in each 24-hour period. Fisher estimated temperature units for all runs in the Sacramento River drainage using average monthly water temperatures at Bend Bridge near Red Bluff. Although growth rate in equation 4 was estimated from juvenile growth up to 90 mm FL, Fisher (1992) used the equation to extrapolate fish growth up to 270 mm FL. Equation 4 and the length-at-date tables based on it have been variously referred to in subsequent reports and interdepartmental correspondence as Frank's Model, the Fisher Model, the DFG Model and the original DFG Model. In this document it will hereafter be called the Fisher Model.

When Fisher issued a draft report describing the Fisher Model in June of 1992, the length-at-date table based on his model had already been in use for several months to estimate winter-run take from salvage data at the SWP and CVP facilities. However, the original Fisher Model length-at-date table only provided size criteria at bimonthly intervals (Figure 2). These size criteria were not averages for the entire first and last half of each month, but rather discrete estimates of size ranges for the four Chinook salmon runs at the beginning and midpoint of each month. The classification of Chinook salmon encountered between these dates could be ambiguous. For instance, a 47 mm Chinook salmon captured on December

7 (between dates with size criteria) would be greater than the 45 mm maximum fork-length for spring-run Chinook salmon based on December 1 criteria, but smaller than the 49 mm minimum fork-length for winter-run Chinook salmon by based on December 16 criteria. Ambiguous fork-lengths such as this created overlap categories between run classification boundaries (Figure 3). Chinook salmon with fork-lengths falling within these overlap categories were double classified (e.g. spring,-winter-run).



Chirock Salmon Spouning in Upper Sacrameuro River and Fry emergence.
Figure 1 Fisher's original length-at-date chart for Chinook salmon in the upper Sacramento River from Stevens (1989).

## DATA FROM TCFFOUT.WK1 REGRESSION GROWTH CURVES FOR INDIVIDUAL RACES

(MM FL.)												
SPAWNING	FALL RUN			L.FALL RUN			WINTER RUN			SPRING RUN		
	EARLY	PEAK	LATE	EARLY	PEAK	LATE	EARLY	PEAK	LATE	EARLY	PEAK	LATE
TIME	OCT1		DEC31	JAN1		APR15	APR16		AUG15	AUG16		SEP30
EMERGE	DEC10		APR2	APR3		JUN27	JUN28		OCT18	OCT19	1	DEC9
DEC	34			166	122	89	89	65	45	45	41	34
mid month	37			181	136	99	99	73	49	49	45	37
JAN	41			200	150	110	110	80	54	54	49	41
	45			219	166	122	122	89	59	59	54	45
FEB	49	34		244	181	136	136	99	65	65	59	49
	54	37		270	200	150	150	110	73	73	65	54
MAR	59	41			219	166	166	122	80	80	73	59
	65	45			244	181	181	136	89	89	80	65
APR	73	49	34	34	270	200	200	150	99	99	89	73
	80	54	- 37	37		219	219	166	110	110	99	80
MAY	89	59	41	41		244	244	181	122	122	110	89
	99	65	45	45	34	270	270	200	136	136	122	99
JUN	110	73	49	49	37			219	150	150	136	110
	122	80	54	54	41			244	166	166	150	122
JUL	136	89	59	59	45	34	34	270	181	181	166	136
	150.	99	65	65	49	37	37		200	200	181	150
AUG	166	110	73	73	54	41	41		219	219	200	166
	181	122	80	80	59	45	45	34	244	244	219	181
SEP	200	136	89	89	65	49	49	37	270	270	244	200
	219	150	99	99	73	54	54	41			270	219
OCT	244	166	110	110	80	59	59	45				244
	270	181	122	122	89	65	65	49	34	34	.,	270
NOV		200	136	136	99	73	73	54	37	37	34	
		219	150	150	110	80	80	59	41	41	37	
DEC		244	166	166	122	89	89	65	45	45	41	. 34
		270	181	181	136	99	99	73	49	49	45	37
JAN	İ		200	200	150	110	110	80	54	54	49	41
		-	219	219	166	122	122	89	59	59	54	45
FEB	l		244	24.4	181	136	136	99	65	65	59	49
=			270	270	200	150	150	110	73	73	65	54
MAR					219	166	166	122	80	80	73	59
100					244	181	181	136	89	89	80	65
APR ·					270	200	200	150	99	99	89	73
1411/		****				219	219	166	110	110	99	80
MAY						244	244	181	122	122	110	89
II IKI				·		270	270	200	136	136	122	99
JUN								219	150	150	136	110
JUL								244	166	166	150	122
								270	181	181	166	136
A110									200	200	181	150
AUG									219	219	200	166
055	VEDICIE	- A - I -	00.00	D 0/00'5			<u> </u>		244	244	219	181
SEP	VERIFIE						/A: C::	~ ~-	270	270	244	200
COT	SLOPE								)			219
OCT	CORRE						HATCHIN	IG.				244
	SIZE RA	NGE U	<b>LON H</b>	TCHING	i 28–35	MM.				<u> </u>		270

Figure 2 Original bimonthly length-at-date table from Fisher (1992). For each date, fork length thresholds are read in a row from right to left. For each run, the fork length on the right is the minimum size expected for that run, representing late-spawned, late-emerged Chinook salmon; the fork length on the left represents maximum size or earliest-spawned Chinook salmon.

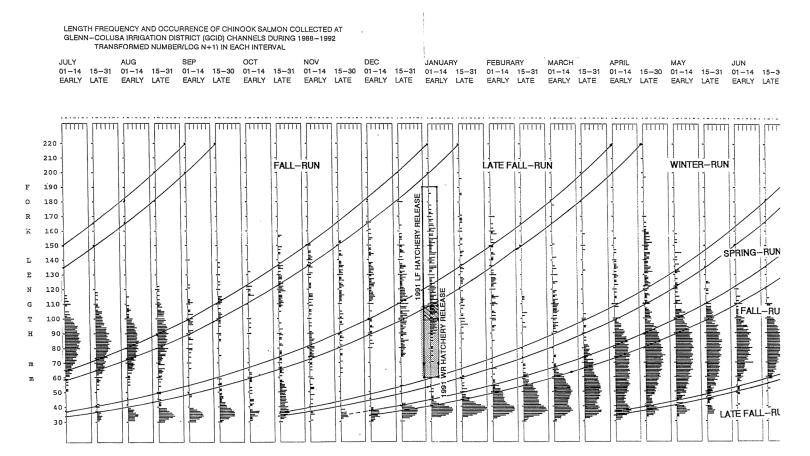


Figure 3 Length at Date boundaries based on Fisher Model bimonthly interval table showing overlap areas between runs. Figure from Fisher (1992).

#### Additional Refinements

By early 1992, Department of Water Resources (DWR) was using Fisher's original bimonthly table to estimate winter-run take at the Delta pumping facility. At the same time, DFG was estimating winter-run take at the Delta pumping facility using a similar table produced by the Fisher Model, but with monthly rather than bimonthly intervals. The larger time intervals in the monthly table resulted in larger overlap zones and caused discrepancies between DWR and DFG take estimates. To alleviate these discrepancies and forestall the possibility of disagreements about whether or not to include double-classified Chinook salmon in take estimates. Sheila Greene (DWR) created a daily-interval table with no overlap categories (Greene 1992). To create the table, Greene fitted a log-linear regression equation to the bimonthly size boundaries in Fisher's bimonthly table,

$$ln(FL,mm) = 3.49470 + 0.0065678*days$$
 (5)

and then used this equation to interpolate daily size thresholds between bimonthly points. Comparison with Fisher's (1992) original fork-length growth equation (equation 4) shows equation 5 is effectively a reproduction of the Fisher Model equation with small differences in parameter values. Since 1992, Greene's daily-interval version of the Fisher Model length-at-date table has been used to designate Chinook salmon juvenile run-origin in the Sacramento River, although the name has been changed from the Fisher Model to the "River Model." Greene's daily-interval table was also used to designate run-origin in the Delta and to estimate winter-run Chinook salmon take at SWP and CVP facilities until it was replaced by the "Delta Model" in April, 1997.

The Delta Model, along with a Modified Fisher Model (aka modified DFG Model), a separate USFWS model developed from the same data as the Fisher Model, and the Seine Model, were all developed in 1994 by a subcommittee of the interagency Winter-run Monitoring and Loss Group. The interagency subcommittee, dubbed the Size Criteria Group, was established in response to a memorandum sent from the director of DWR to the Governor's Water Policy Council (Gibbons 1994), a council comprised of directors of state departments and secretaries of state agencies with a direct interest in state water policy. The memorandum questioned both the validity of

Fisher Model size criteria used to designate Chinook salmon juvenile run-of-origin and the true identity of Chinook salmon salvaged at the SWP fish protection facility that were designated winter-run using the Fisher Model size criteria. To support this criticism, the memorandum stated that coded-wire tagged fall-run Chinook, originating from a hatchery, had been salvaged at the SWP fish facility and misclassified as winter-run Chinook by Fisher Model size criteria. The Size Criteria Group was tasked with modifying or replacing the Fisher Model to produce a new model for predicting Chinook salmon run-origin using length-at-date data in the Delta. The new model was expected to generate size criteria that better differentiated winter-run Chinook salmon juveniles in the Delta from juveniles of other runs, primarily the fall-run. More specifically, the fork-lengths separating winter-run from other runs at any given date were expected to be higher relative to the Fisher Model, so that fewer Chinook salmon at the upper end of the fall-run size distribution would be classified as winter-run and included in winterrun take. This objective stemmed from a suspicion among Size Criteria Group scientists that winter-run growth rate in the Sacramento River is greater than the fall-run growth rate, based on speculation that higher water temperatures during winter-run residence in the Sacramento River cause faster growth rates (Holsinger 1995). Size Criteria Group members also believed juvenile growth rates of all Chinook salmon runs in the Delta are greater than in the Sacramento River (Holsinger 1995). The primary evidence of faster Delta growth rates was a study by Size Criteria Group member Martin Kjelson (1982), which compared growth rates between the upper Sacramento River and Delta, using mark-and-recapture of fall-run hatchery fry.

The Size Criteria Group developed the four alternatives to the Fisher Model over the next several months. The changes in the winter-run length-at-date boundaries projected by the alternative models are projected in Figure 4. Fisher, also a member of the Size Criteria Group, presented a modified version of his original model that simply raised the intercept of the growth equation (equation 4) from average observed emergence size (34 mm) to maximum observed emergence size (41 mm), while retaining the same growth rate obtained from the Tehama-Colusa Canal spawning channel. The modified Fisher Model met the objective of raising the winter-run lower size threshold, but also raised all the other size thresholds, which was not supported by length-frequency data from the Delta (Holsinger 1995). The USFWS conducted an

independent analysis of Tehama-Colusa Canal fish data to determine new growth rates for estimating winter-run size criteria (USFWS 1994). This analysis was identical to Fisher's (1992) original analysis, except upper and lower 95% confidence limits were calculated for the count-perbulk-weight to condition-factor conversion equation. These confidence limits were propagated through the

remaining Fisher Model conversions and calculations to provide 95% confidence limits for growth rate, which were in turn used to determine winter-run upper and lower size criteria.

# OBSERVED CHINOOK SALVAGE AT THE SWP & CVP DELTA FISH FACILITIES 8/1/94 THROUGH 5/31/95

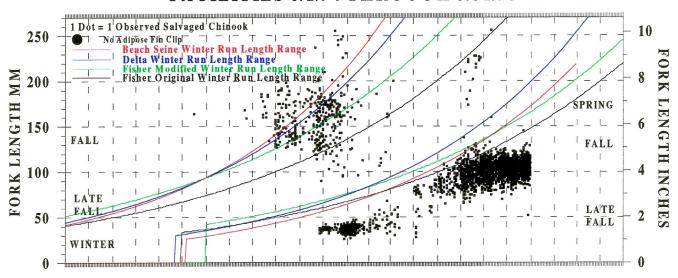


Figure 4 Winter-run Chinook salmon length-at-date boundaries projected by the Greene daily-interval version of the original Fisher Model, and three alternative models developed by the Size Criteria Group in 1994. Delta Model size criteria are from Mark Pierce's original Delta Model length-at-date table. Figure from Greene (1995).

A third alternative, offered by Size Criteria Group member Jay Bigelow (USFWS), was the Seine Model, so named because it was developed from analysis of Chinook salmon length-frequency histograms from beach seine sampling at 14 sites in the upper Sacramento River (RM 164 to 300) over the 14 year period from 1980 to 1994 (Bigelow 1994). The stated goal of Bigelow's analysis was to develop size criteria for winter-run Chinook salmon in the Delta from length-frequency data. Daily seine data was separated into distinct clusters with an objective algorithm. Clusters were pooled across years, but separated by site and day of the year, and then assigned to either winter or fall-run through an iterative regression process. In the iterative process, each length-frequency cluster was initially assigned to a run based on

Fisher Model size criteria. For each run at each site, separate regressions lines were generated from cluster maximum and minimum fork-lengths (cluster tops and bottoms) intercepts taken from the Fisher Model (fork-length = 34 mm at earliest and latest emergence dates), except that emergence date ranges for fall and spring-runs were lumped together for fall-run. The outer 95% confidence intervals of these regression lines were used to reassign clusters to run. The regression process was repeated until consecutive regressions yielded the same parameters. Then for each run, clusters (based on final designation) were pooled across sites and a final regression was conducted for cluster tops and bottoms. The outer 95% confidence intervals of the final regression lines were used to establish length-at-date criteria for each run. Since

data limitations only allowed estimation of winter-run and fall-run boundaries, fork-lengths between the upper winter-run and lower fall-run boundaries were assigned to late fall-run.

To determine if length-at-date patterns from the upper Sacramento River differ from patterns in the lower Sacramento River and Delta, Bigelow (1994) attempted similar analyses for beach seine data collected from these regions. Although length-frequency clusters were apparent for most lower Sacramento River and Delta locations, there was not sufficient sampling during the winter months to develop regressions from the length-frequency clusters. However, Bigelow found length-frequency clusters from lower Sacramento River locations fit reasonably well within upper Sacramento River size criteria, while forklength clusters from most Delta locations suggested fall-run growth rates were higher in the Delta.

The Delta Model, created by Mark Pierce (USFWS) was essentially Greene's daily-interval length-at-date table, based on the original Fisher Model, with modified upper and lower boundaries for winter-run Chinook salmon. The modified winter-run size criteria were determined from length-frequency histograms compiled for non-adipose clipped Chinook salmon from sampling efforts throughout the Delta from 1973 to 1994. This pooled data set was comprised of 140,087 records including USFWS beach seine data (1976-1993), data supplied by Ray Shafter of DFG (10,000 records collected year round, 1973-1974), USFWS trawling data from Sacramento and Chipps Island (1991-1993) and Montezuma Slough (1992-1993), fyke net data from Sacramento (1992-1993), rotary screw trap data from the Sacramento Cross Channel (1993), push net data (1993) and salvage data from the CVP and SWP south Delta fish facilities (1980-1994). Data was pooled across years but separated at bimonthly intervals (e.g. early December, late December). For each bimonthly period a lower boundary for winter-run fork-length was selected by hand from the apparent break between fall-run and winter-run clusters in the length-frequency histograms. Although there were a large number of records in the dataset, the analysis of length-frequency histograms produced only thirteen breakpoints. The natural log of these thirteen break points were regressed against day of the year to obtain an equation representing the lower fork-length boundary for winter-run Chinook salmon in the Delta.

$$ln(FL) = 3.401 + 0.008157*days$$
 (6)

Day = 0 was set at October  $12^{th}$ . Upper boundary break points for winter-run were not clear in the lengthfrequency histograms. Therefore the upper length-at-date boundary was estimated as a line with the same slope as the lower boundary, but with an intercept point set at the largest size (94 mm) of winter-run Chinook salmon entering the Delta, as predicted by the Seine Model, with November 1 as the assumed earliest entry date of winterrun juveniles into the Delta. The regression equations were then used to replace the size criteria for winter-run Chinook salmon in Greene's daily-interval length-at-date table, leaving the Fisher Model size criteria for the other runs unchanged. Sizes below 94 mm for the upper boundary were extrapolated backward to 34 mm with a slightly reduced regression slope of 0.0081, allowing day = 0 to fall on July 1. For models based on Sacramento River growth rates, day = 0 corresponds to assumed earliest and latest emergence dates for a given run. This is not the case for the Delta Model. Instead, the date when day = 0 for the Delta Model boundary equations corresponds to the date when back-extrapolated fork-length equals the average observed emergence size for fall-run Chinook salmon taken from Fisher (1992). Since Chinook salmon forklengths are expected to correspond to Delta Model growth rate and size categories only upon entering the Delta, the date in the Delta Model length-at-date table where day = 0 is somewhat meaningless in the context of Chinook salmon life stage.

The concluding report of the Size Criteria Group recommended the Seine Model be adopted in the Sacramento River and either the Seine Model or Delta Model be adopted in the Delta for designating juvenile Chinook salmon run-origin. The group reasoned these models were more protective for identifying winter-run Chinook salmon because of a broader winter-run size range than the other models (Holsinger 1995). The group found the original and modified Fisher models and the USFWS model were unreliable for estimating run-origin of juvenile Chinook salmon in the Delta because, like the original Fisher Model, they were based on the questionable assumptions that. 1) fish in the Tehama-Colusa artificial spawning channel grow at the same rate as fish in the Sacramento River, and 2) juveniles of all races grow at the same rate as fall-run, even though juveniles of each run, at a given developmental stage, experience different environmental conditions (e.g. temperature, food availability) due to different emergence times and migration patterns.

The group considered the Seine Model more "biologically valid" than the Delta Model for use in the Delta

because, 1) the Seine Model separately estimated size criteria for winter-run and fall-run, while the upper boundary of winter-run size criteria in the Delta Model was derived from a growth rate based on the upper boundary of fallrun size criteria, 2) the length-frequency data used to develop the Seine Model contained a large proportion of winter-run Chinook salmon, while the Delta Model dataset contained a small proportion of winter-run Chinook salmon relative to fall-run Chinook salmon, 3) Seine Model size criteria were developed from objective assessment of length-frequency data and encompassed 95% confidence intervals, while the designation of size thresholds for the Delta Model were somewhat arbitrary, with difficult-to-distinguish breaks between length-frequency clusters drawn in by hand, and 4) the Delta Model suggested slower maximum growth of winter-run in the Delta than the Seine Model suggested in the upper Sacramento River, which ran contrary to the group's expectation that growth would be more rapid in the productive Delta. The report suggests that the Delta Model may become more robust and a better choice in the future as more data is collected in the Delta.

Following completion of the subcommittee report, NMFS did not revise the CVP/SWP biological opinion to implement any of the alternative models for salvage and loss estimates. NMFS was concerned that the models, which were primarily focused on excluding the April/May pulse of fall-run juveniles from winter-run size criteria, did not adequately address size criteria separating the other Chinook salmon runs, particularly earlier in the juvenile migratory season (December - March). However, on March 25, 1997, DWR notified NMFS that the Delta export facilities exceeded 1% of that year's estimated population (Hogarth 1997). When the 2% take limit was exceeded the following day, on March 26, 1997, NMFS initiated interagency discussions to review take estimation procedures. On April 7, 1997, NMFS issued a letter to DWR and the U.S. Bureau of Reclamation implementing replacement of the Fisher Model with the Delta Model for estimation of winter-run take at the Delta export facilities, to be applied retroactively to estimate take for the entire 1996-1997 juvenile out-migration season (Hogarth 1997). The revised take estimate using Delta Model size criteria fell well below the 2% of population limit.

To support the decision to replace the Fisher Model with the Delta Model, the NMFS letter outlined the following conclusions of its review of take estimation procedures:

- The Fisher Model represents Chinook salmon growth rates in the upper Sacramento River and should not be expected to adequately distinguish winter-run Chinook salmon from the other runs in the Delta where growth rates may be higher.
- Chinook salmon in the Fisher Model winter-run size criteria were collected in the San Joaquin River where no winter-run occurs.
- The pulse of juvenile Chinook salmon that was responsible for the exceedance of take had begun in late March when historical records suggest most winter-run should have already completed seaward migration.
- Most Chinook salmon causing exceedance of take by Fisher Model criteria were near the lower size threshold for winter-run and appeared to be part of a large population that fell mostly within fall-run size criteria.
- Preliminary results for newly developed diagnostic genotypes indicated many fall-run at the large end of the population size distribution were wrongly designated winter-run by Fisher Model size criteria.

There was no indication in the letter why the Delta Model was chosen over the Seine Model.

Although initially adopted only for the 1996-1997 season, following a more thorough review of salmon emigration data and genetic analyses provided by researchers at Bodega Marine Lab, NMFS authorized continued use of the Delta Model for take estimation at Delta water export facilities. The length-at-date table in current use at the CVP and SWP Delta export facilities is essentially the same table adopted for use in April 1997, with minor differences in winter-run upper-boundary size criteria corresponding to a slight reduction in the upper boundary regression slopes. Slope reductions were from 0.0081 to 0.008 for size criteria before November 1, and from 0.00816 to 0.00806 for size criteria after November 1. The maximum change in winter-run size criteria caused by this slope change was 2 mm, occurring at the earliest appearance of winter-run in the Delta Model length-at-date table. Most likely, these minor changes were caused by rounding errors during reproduction of the length-at-date table over the years.

#### Discussion

When reviewing the history of size criteria development and implementation it is important to remember that resource managers were faced with a rapidly declining population of winter-run Chinook salmon and required an immediate method for distinguishing juvenile Chinook run origin within a mixed population. The method needed to be fast and simple enough to allow near real-time assessment of winter-run take at state and federal water export facilities, yet provide a level of accuracy that would minimize misclassification of run-origin. The economic, political and ecological implications of inaccurate classification were (and still are) enormous. Winter-run misclassified as non-winter-run could jeopardize survival of the run, while misclassification in the other direction could lead to erroneous curtailment of water exports. Resource managers adopted the length-at-date classification approach and associated size criteria because it was the best available science at the time.

Over the years, with continued use, Fisher Model and Delta Model size criteria have become established standards, even while knowledge of the origins of the criteria have slipped into obscurity. As a result, few (if any) practitioners currently using the length-at-date approach are aware of the tenuous assumptions and disjointed or limited datasets used to develop the size criteria. Fortunately, new classification approaches are under development or consideration. These new approaches range from rapid, real-time DNA analysis, to fine-scale evaluation of morphological characteristics, to analyses of multiple environmental variables to predict arrival of juvenile Chinook salmon pulses at pumping facilities. All of these approaches share a reliance on accurate DNA-typing of the individual fish used for model parameterization. Any one of these approaches has the potential to provide a more rigorous and dependable means for assessing the run origin of juvenile Chinook salmon encountered in the Sacramento River and the Delta.

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U.S. Environmental Protection Agency

National Marine Fisheries Service

BEFORE CITING INFORMATION HEREIN, CONSIDER THAT ARTICLES HAVE NOT RECEIVED PEER REVIEW.